nsls
IR UV X

Science Highlights

from the National Synchrotron Light Source

BEAMLINE
X26A

PUBLICATION
J.A. Howe, R.H. Loeppert, V.J. DeRose, D.B. Hunter and P.M. Bertsch, "Localization and Speciation of Chromium in Subterranean Clover using XRF, XANES, and EPR Spectroscopy," *Environ. Sci. Technol.*, **37**, 4091 (2003).

FUNDING
DOE, Environmental Remediation Sciences Div. in the Office of Biological and Environmental; U.S. DOE and the University Research Alliance, formerly the Amarillo National Research Center

FOR MORE INFORMATION
Richard H. Loeppert, Soil and Crop Sciences Department, Texas A&M Univ.
r-loeppert@tamu.edu

Localization and Speciation of Chromium in Subterranean Clover Using XRF, XANES, and EPR Spectroscopy

Julie A. Howe[1], Richard H. Loeppert[1], Victoria J. DeRose[2], Douglas, B. Hunter[3], and Paul M. Bertsch[3]

[1]Department of Soil and Crop Sciences, Texas A&M University; [2]Department of Chemistry, Texas A&M University; [3]Advanced Analytical Center for Environmental Sciences, Savannah River Ecology Laboratory, University of Georgia

Scientists from Texas A&M University and the University of Georgia have used X-ray absorption near edge spectroscopy (XANES) and synchrotron X-ray fluorescence (SXRF) microprobe spectroscopy at NSLS beamline X26A, as well as electron paramagnetic resonance (EPR) spectroscopy, to investigate the absorption, localization, and transport of chromium (Cr) in plants. These studies have shown that the reduction of Cr(VI) to Cr(III) and the immobilization or compartmentalization of Cr are important detoxification mechanisms used by the plant.

The industrial use and release of chromium compounds into the environment has led to the contamination of water and soil, and therefore to associated environmental and health concerns. In soils, Cr occurs predominately in the +3 and +6 oxidation states. The more soluble and more toxic +6 state presents the greater environmental concern. In efforts to remediate soils contaminated with low levels of Cr(VI), the use of plants to extract or immobilize the metal (phytoremediation) has been proposed. But before phytoremediation can be performed effectively and safely, it is necessary to understand the mechanisms of Cr uptake, translocation, tolerance, and bonding by plants, and the conditions under which Cr is absorbed and/or immobilized.

Nondestructive techniques, such as electron paramagnetic resonance (EPR) spectroscopy, X-ray absorption near edge spectroscopy (XANES), and synchrotron X-ray fluorescence (SXRF) microprobe spectroscopy, are useful for the chemical investigation of Cr, to minimize changes in the metal's chemistry that might occur with destructive techniques. The objectives of this study were to localize Cr in the leaf using SXRF microprobe spectroscopy, determine the oxidation state of Cr in the plant using EPR and XANES, and evaluate possible modes of Cr complexation in the plant using EPR. These analyses were performed on subterranean clover plants exposed to various concentrations of inorganic Cr(III), Cr(VI), and Cr(III)-organic complexes.

Localization of the translocated Cr in leaves using SXRF microprobe spectroscopy indicated two distinct Cr accumulation patterns. At the low Cr(VI) treatment concentration (0.04 mmol L^{-1}), Cr accumulated primarily at leaf margins with some slight accumulation in the veins (**Figure 1A**). This result corresponded with visual observations of red pigmentation at the leaf margin. At the high Cr(VI) treatment concentration (1.6 mmol L^{-1}), Cr accumulation was observed in or around the veins (**Figure 1B**). This accumulation corresponded to regions of brown coloration and tissue damage along the leaf veins. The high Cr(VI) concentration resulted in the death of the plants within seven to 10 days.

Coauthors (left) Vickie DeRose, Julie Howe, and Richard Loeppert

The oxidation state of Cr in the plant was determined using both EPR and XANES. This combination of techniques was used because the +6 oxidation state is not detectable using EPR, but is easily identifiable as a pre-edge peak in the Cr K-edge XANES spectrum. In plants grown with the low Cr(VI) treatment concentration and with various Cr(III) treatments (i.e., $CrCl_3$, Cr(III)-EDTA, Cr(III)-citrate, and

Cr(III)-oxalate), the Cr in the plant was only observed in the +3 oxidation state. Furthermore, the EPR spectra indicated the occurrence of Cr(III)-organic complexes, except in the case of the plants grown in $CrCl_3$ and the low Cr(VI) treatment. The EPR spectra from these two treatments indicated the presence of a precipitated $Cr(OH)_3$ phase in or on the roots. EPR spectra of both the roots and shoots of plants grown in a high Cr(VI) treatment concentration revealed distinct Cr(V) signals and additional signals of Cr(III)-organic complexes in the leaf. Using XANES, Cr(VI) was only positively identified in the roots of plants grown with the high Cr(VI) treatment concentration (**Figure 2**). Successive scans revealed the rapid disappearance of this peak, indicating the rapid reduction of Cr(VI) (**Figure 2F,G**).

Results from this study provide information on how plants tolerate Cr(VI). This tolerance must involve the complete reduction of highly toxic Cr(VI), Cr(V), or Cr(IV) to the considerably less toxic Cr(III), and either the immobilization or compartmentalization of Cr(III). Three processes of immobilization and compartmentalization were identified: (i) the precipitation of a Cr(III) hydroxide phase at the root, (ii) the complexation of Cr(III) and its probable storage as Cr(III)-organic complexes, and (iii) the transport of Cr(III)-organic complexes to the leaf margins, where the Cr is less disruptive to plant metabolic processes.

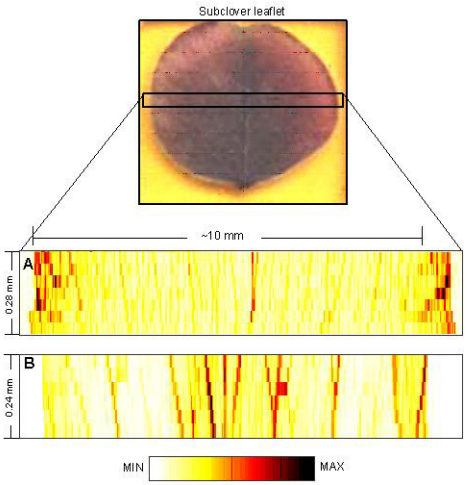

Figure 1. *SXRF microprobe images of the relative Cr concentrations in transects of subclover leaves grown in (A) 0.04 mmol L^{-1} Cr(VI) for 21 days and (B) 1.6 mmol L^{-1} Cr(VI) for 4 days. The rectangle on the subclover leaflet at the top of the figure indicates the approximate location of the transect.*

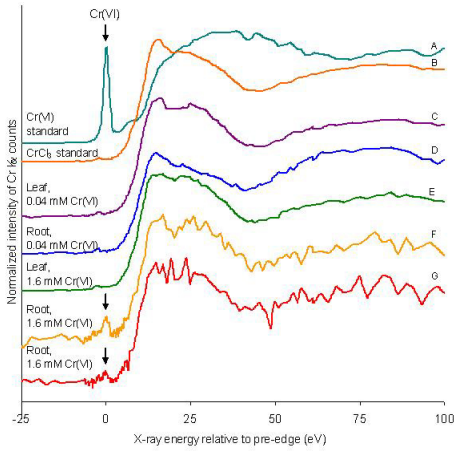

Figure 2. *XANES Cr K-edge spectra of 5% (w/w) standards of (A) Cr(VI) and (B) Cr(III), fresh subclover (C) leaf and (D) root tissue grown with 0.04 mmol L^{-1} Cr(VI), fresh subclover (E) leaf and (F, G) root tissue grown with 1.6 mmol L^{-1} Cr(VI). Spectra F and G are successive scans at the same position on the root indicating the rapid reduction of Cr(VI).*